mosomes of IIIB/*ru h D*^{3} *st ri InRC e l3e* heterozygotes were then examined. A large paracentric inversion was observed in the left arm of the IIIB chromosome with distal and proximal breaks corresponding to those for the Payne inversion (*3*), symbolized by *In(3L)P*.

Genetic evidence for the presence of the Payne inversion in the *tu-h* strain was obtained by crossing IIIB/*ru h D*^{3} *st ri InRC e l3e* flies with those with the markers *R Ly/In(3L)P*, and picking out in the offspring flies that were IIIB/*In(3L)P*. These flies were viable, showing that different lethals are associated with the two chromosomes. Inbreeding these types gave rise to about 30 percent recombinant scarlet offspring, resulting from the freeing of *st* by crossing-over from the lethal or lethals associated with the left arm of the IIIB chromosome. The occurrence of scarlet offspring demonstrates that pairing of the chromosomes in meiosis is normal and, therefore, that the inversion in the IIIB chromosome is *In(3L)P*.

The Payne inversion is widespread in many natural populations and laboratory stocks of *Drosophila melanogaster* (*3*). The interesting problem is what advantage the chromosome containing this inversion bestows upon its bearers in the *tu-h* strain, for it represents a remarkable case of natural selection for the heterozygote in a laboratory population. All *tu-h* strains maintained at the University of Utah contain the IIIB chromosome, and from the results of outcrosses the scarlet gene is known to have been heterozygous in all these strains since at least 1951. The techniques of maintaining the stocks have varied over the years. In some generations flies showing the tumorous-head trait have been selected as parental types; in others, mass mating procedures have been employed. Under either method, and even when the number of parental flies utilized has been small, the IIIB chromosome has been kept at a high frequency. The suppression of crossing-over in the left arm of chromosome III by the Payne inversion has likely led to the establishment of a heterotic mechanism.

Since the *tu-1* gene has been found in the homozygous condition in several different natural and laboratory populations (*4*), it seems likely that the original flies collected in Mexico possessed *tu-1* and were also undergoing segregation for the Payne inversion. The mutations to *tu-3* and *st* occurred later in the laboratory stock, although *st* might have been present on the same chromosome as the inversion in the original flies.

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Appearance of Genetic Transforming Activity in Pneumococcal Cultures

Abstract. Growing populations of *Pneumococcus* were found to release into the culture medium deoxyribonucleate-containing material with genetic transforming activity. Active material was maximally produced at the time when the culture was most responsive to added deoxyribonucleate. Since mixed cultures thus give rise to recombinants, it may be that transformation provides a natural mechanism of genetic recombination for *Pneumococcus*.

In 1944, Avery, MacLeod, and McCarty (*1*) established the genetic transforming principle of *Pneumococcus* to be deoxyribonucleic acid. Since that time most transformations, in pneumococci and in other transformable bacteria, have been carried out with purified soluble deoxyribonucleate, with the principal exception of the lysate transformation system described by Hotchkiss (*2*). In this system, penicillin, or later streptomycin, was used to kill sensitive organisms carrying a suitable genetic marker. If appropriate recipient cells resistant to the drug were mixed with the sensitive population, a fraction of the resistant recipients were transformed by genetic material from the dying population. This method was used mainly as a tool for simplified transformation tests until we recently began investigations on genetic and physiologic aspects of transformation by lysed cells.

The bacteria used in this investigation were pneumococcal strain R_6 [a single-colony derivative of R36A (*1*)] and derived variants genetically resistant to streptomycin, sulfonamide, micrococcin, and amethopterin, singly or in combination. These variants were obtained by transformation of the R_6 wild type with the appropriate deoxyribonucleate extracted from cultures of spontaneously arising resistant mutant strains.

Deoxyribonucleate was prepared by the method described by Hotchkiss (*3*), based on the method of McCarty and Avery (*4*). The bacteria were grown in a casein hydrolyzate medium enriched with vitamins, minerals, glucose, bovine serum albumin, and neopeptone. Cultures to be used as recipients were frozen at −20°C in 10-percent glycerol when they had reached the transformable state (*5*) and maintained at this temperature. The frozen cultures retained their transformability during 2 to 3 months of storage.

Bacteria to be used as donors were freshly grown (starting with an inoculum from a culture thawed at 0°C) on the day of use. Total population size and number of transformants were estimated by colony counts in broth containing specific antibody in the presence of which each viable unit grows as a visible aggregate (*6*). Transformations were initiated by exposing the freshly thawed diluted recipient cells to donor material for 30 minutes at 30°C (*5*). At this time pancreatic deoxyribonuclease was added to terminate the reaction by destroying any deoxyribonucleate not taken up by the cells. The cultures were then incubated at 37° for 30 to 90 minutes to permit expression of the newly acquired traits, after which aliquots were diluted into the appropriate scoring medium. Colonies were counted after 16 to 20 hours' incubation at 37°C. When cells were to be separated from culture fluid, Millipore filters of porosity 0.45 ± 0.02 μ (Millipore Filter Corp.) were used.

During the course of experiments on the mechanism of streptomycin-induced lysate transformation it was found that cell-free filtrates of control cultures not treated with drug, and therefore presumably normal, were often able to effect transformation of recipient cultures. When this phenomenon was investigated further it was found that all of the drug-resistance properties mentioned earlier could be regularly induced in recipients by using filtrates of appropriate living cultures, and that the activity in the filtrates was probably due to some deoxyribonucleate-containing material, since it, like the activity released by drugs, could be destroyed by pancreatic deoxyribonuclease. The deoxyribonucleate in such material was of sufficient intactness to transfer linked determinants of sulfonamide resistance (*6*), the frequency of double transformants being as much as 1000 times that expected on the basis of chance.

It was also found that the amount of active transforming material present in any culture filtrate was related to the stage of growth, the maximum activity appearing at about the middle of the logarithmic phase (about 2×10^7 colony-forming units per milliliter). Because transforming activity and the stage of growth of the culture were clearly related, and because transformability by exogenous deoxyribonucleate also varies with stage of growth of the culture, it was of interest to study the relation between the abilities to serve as

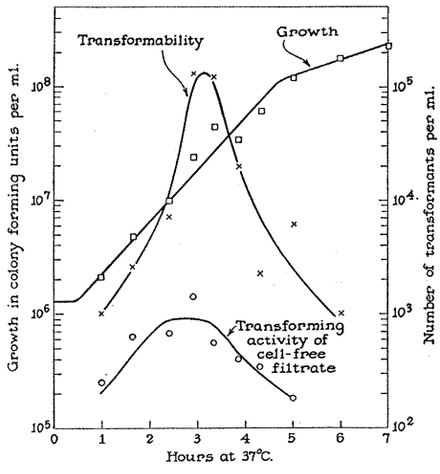

Fig. 1. Transformation by culture filtrates. The transformability of a growing culture of sulfonamide-resistant cells was tested with deoxyribonucleate carrying streptomycin resistance, at the times shown. At the same times, filtrates of the culture were prepared and used to transform sulfonamide-sensitive cells to sulfonamide resistance. On occasion, as many as 1 percent of the cells are transformed by culture filtrates in such experiments. This represents about one-tenth of the maximum yield obtainable by isolated deoxyribonucleate under comparable conditions.

donor and as recipient in the same culture. The results of one experiment are illustrated in Fig. 1. It appears that at a time in growth when a population is producing the greatest quantity of material for transforming other cells, the population is itself most susceptible to transformation.

The correlation in time between production of transforming activity and transformability need not, of course, imply a causal relationship between the two functions, either within the population or in individual cells. We have evidence, however, that with growth there is released into the culture medium some factor which interferes with transformation by added isolated deoxyribonucleate, although it does not clearly behave like a deoxyribonuclease. This effect is being investigated further and may explain in part the reduction in the transforming activity of late filtrates and even the decrease in transformability of pneumococcal cultures allowed to grow beyond a certain point.

The coincidence in time of maximum transformability and maximum production of transforming activity made it feasible to attempt the recovery of recombinants from differently marked cultures grown in each other's presence. The results of one mixed-growth experiment are presented in Fig. 2. Two cultures, one resistant to sulfonamide and amethopterin, the other resistant to streptomycin and micrococcin, were inoculated into the same culture tube. Transformants having resistance to three drugs were scored in aliquots of the culture at different times during growth. Since the determinants, being unlinked, are normally transferred singly, it was possible to detect the direction of transformation. It is evident that each culture was able to donate both of its markers and to accept both markers of the other culture (*7*).

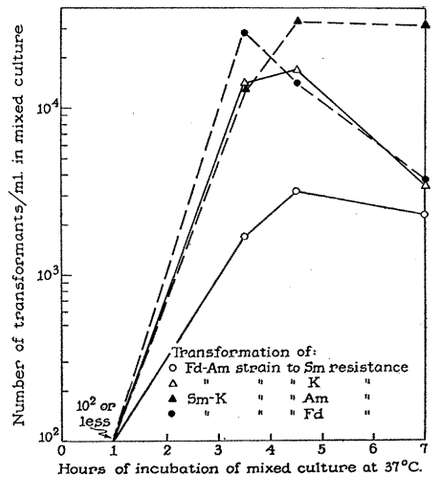

Fig. 2. Transformation in mixed growing populations: sulfonamide (Fd) and amethopterin (Am) resistant strain mixed with streptomycin (Sm) and micrococcin (K) resistant strain.

These experiments indicate that extracellular deoxyribonucleate-containing material can be found in growing pneumococcal cultures. Several authors have reported the accumulation of extracellular deoxyribonucleate in cultures of other bacteria, such as some halophilic bacteria (*8*), *Brucella* (*9*), *Micrococcus* (*10*), *Alcaligenes* (*10*), *Pseudomonas* (*10*). *Flavobacterium* (*10*), and *Neisseria* (*11*). In such cultures, however, deoxyribonucleate accumulated very late in the growth cycle, or else under unfavorable growth conditions, and was produced in such abundance (about 100 mg per liter of culture for *Neisseria*) that the growth became visibly slimy. In the case of *Neisseria meningitidis,* Catlin (*11*) also demonstrated that the extracellular deoxyribonucleate had transforming activity similar to that of deoxyribonucleate extracted from cells. Both Takahashi (*12*) and Catlin (*11*) attributed the accumulation of extracellular deoxyribonucleate to death and lysis of a fraction of the population, and Catlin (*10*) also proposed that the production of significant amounts of an active deoxyribonuclease-inhibitor (a ribonucleic acid) by some cultures might explain why such an accumulation occurred.

The situation in *Pneumococcus,* however, seems to be quite different, because deoxyribonuclease-sensitive material with transforming activity is maximally produced relatively early in growth under presumably favorable conditions and does not accumulate in large amounts. The absence of activity in older pneumococcal cultures and the failure to accumulate deoxyribonucleate may be due to the increased production, with growth, of material which destroys active deoxyribonucleate. Although studies on other organisms, such as *Escherichia coli* (*13*), seem to indicate that cell death is rare during active growth of the population, we have not yet been successful in determining whether for pneumococci the transforming material released by growing cultures comes from population turnover or from active excretion by living cells. Nonetheless, the existence of such material in pneumococcal cultures lends support to the idea that transformation may not be a phenomenon restricted to laboratory conditions. As pointed out in 1951 (*2*), in a mixed population under strongly adverse environmental conditions, lysate transformation might prevent the total loss of the genome of the sensitive organisms present. Now, the presence of transforming material, and the correlation between transforming activity and transformability in growing populations, make it seem quite possible that transformation in *Pneumococcus* may provide a natural mechanism of genetic recombination for an organism in which, so far, no other such mechanism has been found.

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